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Serotype, Antimicrobial Susceptibility, and Pathogenicity of *Erysipelothrix rhusiopathiae* Isolates from Tonsils of Apparently Healthy Slaughter Pigs

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Erysipelothrix rhusiopathiae **was isolated from tonsils of 63 (10.5%) of 600 apparently healthy slaughter pigs in the Kanto area of Japan in February and July 1984. The isolation rate was significantly higher during July than in February. Of these 63 isolates, 34 isolates (54.0%) were serotype 7, 20 isolates (31.7%) were serotype 2, 6 isolates (9.5%) were serotype 6, and 1 isolate (1.6%) each was serotype 11, 12, or 16. All isolates of serotypes 2, 6, 11, 12, and 16 were highly virulent for mice, whereas most isolates of serotype 7 were weakly virulent. In swine, all isolates of serotype 2 were highly virulent, capable of inducing generalized urticarial lesions with depression and anorexia. On the other hand, 37 of 43 isolates of serotypes other than 2 induced no clinical signs, and the remaining 6 isolates induced local urticarial lesions at the site of inoculation in swine. The MIC of dihydrostreptomycin ranged from 1.56 to 100 μg/ml. All of the dihydrostreptomycin-resistant strains belonged to serotype 2. The high virulence of *E. rhusiopathiae* strains of serotype 2 harbored in the tonsils suggests a possible role of such strains in the cause of swine erysipelas. In contrast, members of the other nonvirulent or weakly virulent group, mainly serotype 7 strains, were considered to be resident in porcine tonsils.**

Erysiopelothrix rhusiopathiae is the causative agent of swine erysipelas, which causes great economic loss and continues to be a major problem in swine-producing areas of the world. The clinical signs of swine erysipelas can be divided into three types: acute (septicemia), subacute (urticaria), and chronic (arthritis, lymphadenitis, and endocarditis). At present, strains of *E. rhusiopathiae* are classified into 22 serotypes and type N, which does not produce any precipitating antibody against homologous and heterologous heat-stable extracts in rabbits (17). It is generally known that most isolates from pigs affected with clinical erysipelas fall into serotypes 1 and 2 (21).

Different tissues of apparently healthy pigs have been examined for the presence of *E. rhusiopathiae* by many authors. The preponderance of these isolations has been from the tonsils, although the organisms have been isolated from the intestinal tract, lymph nodes, gall bladder, joints, and bone marrow (1, 2, 9, 10, 12, 19). The serological classification of *E. rhusiopathiae* isolates from tonsils has been described (2, 7, 9). However, their pathogenic characteristics and drug susceptibility are still unclear.

In the present report, we investigated the serotype, antimicrobial susceptibility, and pathogenicity of *E. rhusiopathiae* isolates from the tonsils of apparently healthy slaughter pigs as compared with those of clinical isolates described previously (1, 4–17), and we attempted to clarify the etiological significance of the organisms harbored in the porcine tonsils.

MATERIALS AND METHODS

Tissues. The tonsils were obtained from 600 pigs, weighing approximately 100 kg, selected at Tachikawa slaughterhouse, Tokyo, in February and June 1984. Before removal of the tonsils, the carcass of each pig had been inspected and designated as normal by inspectors from Tama Meat Inspection Office, Bureau of Public Health, Tokyo Metropolitan Government. Tonsils from each pig were placed in a sterile vinyl specimen bag and transported in ice to our laboratory. Tissues were processed for bacteriologic culture immediately upon arrival.

Culture procedures for isolation of *E. rhusiopathiae*. Approximately 1 g of tonsil tissue was chopped into pieces 2 mm in diameter, and the chopped tissue was inoculated into 10 ml of beef infusion (BI) broth (pH 7.6, prepared in our laboratory) containing 0.1% Tween 80, 50 μg of gentamicin per ml, and 500 μg of kanamycin per ml. After incubation for 24 h at 37°C, one loopful of the culture was streaked onto each plate of BI agar (pH 7.6, prepared in our laboratory) containing 0.1% Tween 80, 50 μg of gentamicin per ml, and 500 μg of kanamycin per ml. The agar plates were incubated for 48 h at 37°C and then examined for the presence of typical *Erysipelothrix* colonies.

Identification of isolates. Representative colonies with typical morphologic characteristics of *E. rhusiopathiae* were picked from each plate, seeded into tubes of BI broth, and incubated for 24 h at 37°C. The isolates were identified as *E. rhusiopathiae* on the basis of cellular morphology, typical reactions in triple sugar iron agar slants, "test-tube brush" growth in gelatin, and negative reactions for esculin hydrolysis, catalase, and oxidase as described by Wood (20).

Serotyping. Serotyping of *E. rhusiopathiae* isolates was performed by a method described previously (5, 6, 17). Colonies from a 48-h-old agar plate culture of each isolate were inoculated into BI broth containing 0.1% Tween 80. Incubation was done for 48 h at 37°C, and the culture was centrifuged at 12,000 × *g* for 20 min. The bacterial cells were

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TABLE 1. Isolation of *E. rhusiopathiae* from tonsils of apparently healthy slaughter pigs

Sampling period	No. of samples examined	No. (%) of isolates obtained
February 1984	300	24 (8.0)[a]
July 1984	300	39 (13.0)[a]
Total		63 (10.5)

[a] $P < 0.05$.

washed three times with physiological saline and suspended in distilled water to 1/30 of the original volume. The bacterial suspension was autoclaved for 1 h at 121°C, cooled, and clarified by centrifugation. The supernatant fluid was tested for its reaction with typing sera (rabbit origin) representing serotypes 1 through 22 of *E. rhusiopathiae* in an agar gel double-diffusion precipitation system.

MICs. MICs were determined by standard methods for agar dilution tests in Mueller-Hinton agar (Difco Laboratories) (3). After inoculation, agar plates were incubated for 48 h at 37°C.

Twofold serial dilutions of antimicrobial stock solutions were prepared so that concentrations of antimicrobial agents ranged from 0.025 to 100 μg/ml. The antimicrobial agents used were penicillin G, ampicillin, erythromycin, oleandomycin, oxytetracycline, chloramphenicol, dihydrostreptomycin, kanamycin, and sulfadimethoxine.

Animals. A total of 1,890 4-week-old female mice of the outbred ddY strain were used. They were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan.

Sixty-three female and castrated male Yorkshire swine, purchased from the Minano Agricultural Cooperative Association for Laboratory Animals, Saitama, Japan, were used when they were 3 to 4 months old. They were conventionally farrowed and raised in confinement. Sera of the swine had growth agglutination titers (11) of 8 or below.

Pathogenicity test. Portions (0.1 ml) of serial 10-fold dilutions of BI broth culture of each isolate were injected subcutaneously into each of five mice. At the same time, 0.1 ml of a 10^{-5} dilution was poured onto two petri plates and mixed with BI agar medium containing 0.75% agar. After 48 h of cultivation at 37°C, colonies in BI agar were counted to determine the number of CFU. For determination of the 50% lethal dose (LD_{50}), mortality rates were recorded 14 days after exposure. The LD_{50}s were determined by the method of Kärber (4).

One pig was inoculated intradermally with 0.1 ml of BI broth culture (approximately 10^7 CFU per pig) of one isolate. Clinical signs were observed every day for 14 days after exposure.

RESULTS

Bacterial isolation from tonsils of pigs. *E. rhusiopathiae* was isolated from the tonsils of 24 (8.0%) of 300 apparently healthy slaughter pigs examined in February 1984 and the tonsils of 39 (13.0%) of 300 pigs in July 1984 (Table 1). The isolation rate was significantly ($P < 0.05$, Fisher exact test) higher in July.

Serotypes of isolates. The serotypes of 63 *E. rhusiopathiae* isolates from tonsils of pigs are shown in Table 2. Of 63 isolates, 34 isolates (54.0%) were serotype 7, 20 isolates (31.7%) were serotype 2, 6 isolates (9.5%) were serotype 6, and 1 isolate (1.6%) each was serotype 11, 12, or 16.

Pathogenicity of isolates. Results of the pathogenicity test for 63 *E. rhusiopathiae* isolates are shown in Table 2. All isolates of serotypes 2, 6, 11, 12, and 16 were highly virulent for mice (LD_{50}s of $<10^{2.0}$ CFU). Of 34 isolates belonging to serotype 7, 6 isolates (17.6%) showed LD_{50}s less than $10^{2.0}$ CFU, 2 isolates (5.9%) showed LD_{50}s ranging from $10^{2.1}$ to $10^{4.0}$ CFU, 20 isolates (58.8%) showed LD_{50}s ranging from $10^{4.1}$ to $10^{6.0}$ CFU, and 6 isolates (17.6%) showed LD_{50}s of more than $10^{6.1}$ CFU.

In swine, all isolates of serotype 2 induced generalized urticarial lesions with depression and anorexia after intradermal inoculation. Of 43 isolates belonging to serotypes other than 2, 6 isolates (14.0%) induced local urticarial lesions at the site of inoculation, and the remaining 37 isolates (86.0%) induced no clinical signs in swine.

Antimicrobial susceptibility of isolates. The MICs for 63 *E. rhusiopathiae* isolates are summarized in Table 3. All isolates were highly susceptible to penicillin G, ampicillin, erythromycin, and oleandomycin (MICs of 0.025 to 1.56 U/ml or μg/ml) and moderately susceptible to oxytetracycline and chloramphenicol (MICs of 3.13 to 25 and 1.56 to 25 μg/ml, respectively). Kanamycin and sulfadimethoxine showed no activity against the isolates. The MICs of dihydrostreptomycin presented two distribution peaks; of 63 strains, 12 (19.0%) were resistant to dihydrostreptomycin (MIC of ≥100 μg/ml). The relationship between susceptibility patterns to dihydrostreptomycin and serotypes of isolates is shown in Table 4. All of the dihydrostreptomycin-resistant strains belonged to serotype 2. Resistance to dihydrostreptomycin was not found in the remaining isolates of serotypes 6, 7, 11, 12, and 16.

TABLE 2. Serotype and pathogenicity of the 63 *E. rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs

Serotype	No. (%) of isolates	Pathogenicity for mice (no. of strains with the indicated log LD_{50})[a]				Pathogenicity for swine (no. of strains inducing the indicated response)		
		≤2.0	2.1–4.0	4.1–6.0	≥6.1	Generalized erythema[b]	Localized erythema[c]	No clinical sign[d]
2	20 (31.7)	20	0	0	0	20	0	0
6	6 (9.5)	6	0	0	0	0	3	3
7	34 (54.0)	6	2	20	6	0	1	33
11	1 (1.6)	1	0	0	0	0	1	0
12	1 (1.6)	1	0	0	0	0	0	1
16	1 (1.6)	1	0	0	0	0	1	0

[a] Mice were inoculated subcutaneously with serial dilutions of broth culture of each strain. LD_{50}s are expressed as the number of viable bacteria per mouse.
[b] Induced generalized erythema with profound depression and anorexia in swine after intradermal inoculation with 0.1 ml of broth culture (approximately 10^8 CFU) of each strain.
[c] Induced localized erythema of ≥20 mm in diameter at the skin injection site.
[d] Induced no clinical sign of erysipelas in swine.

TABLE 3. Distribution of MICs for the 63 *E. rhusiopathiae* strains

Antimicrobial agent	No. of strains with the following MIC (μg/ml):													
	0.025	0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
Penicillin G[a]	8	28	27											
Ampicillin	11	23	29											
Erythromycin		11	26	26										
Oleandomycin				1	4	38	20							
Oxytetracycline								38	2	21	2			
Chloramphenicol							1	4	13	44	1			
Dihydrostreptomycin							3	4	8	32	1	3	7	5
Kanamycin														63
Sulfadimethoxine														63

[a] Penicillin G MICs are given in units per milliliter.

DISCUSSION

It is well documented that the prevalence of *E. rhusiopathiae* carrier pigs among those examined ranges from 3 to 98%, with most surveys indicating that 20 to 40% of pigs are carriers (2, 8, 9, 13, 18). Carrier pigs have been reported among vaccinated as well as nonvaccinated swine (10). In this study, *E. rhusiopathiae* was isolated from tonsils of 63 (10.5%) of 600 apparently healthy slaughter pigs, and there was a tendency for the isolation rate to be higher during the warmer month. A similar variation in isolation rate was described by Murase and Ebi (9) and Timoney (18).

The present investigation demonstrated the presence of a wide variety of serotypes of *E. rhusiopathiae* in tonsils of apparently healthy pigs. Our previous attempts to determine the serotypes of 300 *E. rhusiopathiae* isolates from pigs with various clinical types of erysipelas had shown that 71 isolates (23.7%) were serotype 1a, 18 isolates (6.0%) were serotype 1b, 191 isolates (63.7%) were serotype 2, and 20 isolates (6.7%) were serotype 3, 5, 6, 8, 11, or 21 or type N (17). In contrast, more than half of the isolates from tonsils of apparently healthy pigs surveyed in the present investigation belonged to serotype 7, followed by serotype 2 (31.7%), serotype 6 (9.5%), and serotype 11, 12, or 16 (1.6% each). This finding would indicate some differences in the distribution of serotypes between the isolates from tonsils of apparently healthy pigs and the isolates from pigs with erysipelas.

The pathogenicity of *E. rhusiopathiae* strains frequently isolated from tonsils of apparently healthy pigs is not well known. Their role in the etiology of swine erysipelas also has not been clearly established. In the present study, it was clarified that all isolates of serotype 2 from tonsils were highly virulent for swine, capable of inducing generalized urticarial lesions with depression and anorexia. On the other hand, most isolates of serotypes other than 2 were nonvirulent or weakly virulent for swine and mice. Rowsell (H. C. Rowsell, Proc. 92nd Annu. Meet. Am. Vet. Med. Assoc., p. 143–148) suggested that the tonsils were the initial locus of naturally occurring *Erysipelothrix* infection in swine, followed by invasion of vascular or lymphatic systems. Therefore, the high virulence of *E. rhusiopathiae* strains of serotype 2 harbored in the tonsils supports a possible role of such strains in the cause of swine erysipelas. In addition, it is likely that the existence of highly virulent organisms in the tonsils of carrier pigs not only could represent a portal of entry for primary infection but also would be a persistent source of soluble or particulate *Erysipelothrix* antigens in chronically infected pigs (13). In contrast, the other nonvirulent or weakly virulent strains, mainly serotype 7, were regarded to be resident in porcine tonsils.

The present results on antimicrobial susceptibility of the *E. rhusiopathiae* isolates from porcine tonsils are in general agreement with previous reports on antimicrobial susceptibility of the clinical isolates (14, 15). It should be noted that the MIC of dihydrostreptomycin widely ranged from 1.56 to 100 μg/ml, and all of the resistant strains belonged to serotype 2. Our previous results on isolates from pigs with chronic erysipelas had also showed that most of the strains resistant to dihydrostreptomycin or oxytetracycline belonged to serotype 2 (15). In Japan, pigs are usually fed food containing various antibiotics for the purpose of growth stimulation. It seems, therefore, that long-term administration of antibiotics gives a selective advantage to such antibiotic-resistant strains of *E. rhusiopathiae*.

The results of the present investigation suggest that the cluster of virulent strains of serotype 2 differs from the cluster of avirulent strains of serotype 7 in susceptibility to the drug. This observation on phenotypic characteristics indicates the possibility that the avirulent cluster would be genetically distinct from *E. rhusiopathiae* characterized to date. Therefore, further investigations with chemotaxonomic techniques such as DNA homology would be useful to clarify the genetic relationship among them.

TABLE 4. Comparison between susceptibility of the *E. rhusiopathiae* strains of serotypes 2 and 7 and the others to dihydrostreptomycin

Serotype	No. of strains with the following MIC of dihydrostreptomycin (μg/ml):							
	1.56	3.13	6.25	12.5	25	50	100	>100
2				6	1	1	7	5
7	3	4	7	19		1		
Other[a]			1	7		1		

[a] Includes serotypes 6, 11, 12, and 16.

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